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Parkin and Hsp70 Sacked by BAG5

Loss-of-function mutations in the parkin gene, which encodes an E3 ubiquitin ligase, are the major cause of early-onset Parkinson's disease (PD). In this issue of *Neuron*, Kalia et al. show that the bcl-2-associated athanogene 5 (BAG5) enhances dopamine neuron death in an in vivo model of PD through inhibiting the E3 ligase activity of parkin and the chaperone activity of Hsp70.

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by impairment in motor function including bradykinesia, rest tremor, rigidity, gait abnormalities, and postural instability. Pathologically, PD is marked by the selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the presence of Lewy bodies, intracellular proteinaceous inclusions (Dawson and Dawson, 2003). Because dopaminergic inputs from the SNc are important regulators of corticostriatal neuronal transmission, it is believed that such loss leads to the symptoms of PD through the ultimate impairment of movement-related activation of the prefrontal motor cortex. The molecular mechanism of dopaminergic neuronal cell death is not fully understood, but both genetic and environmental components are believed to contribute significantly to the neurodegenerative process (Dawson and Dawson, 2003).

In recent years, research in PD has been fueled by the identification of genes that are linked to rare Mendelian forms of PD. So far, 11 loci have been described, and 5 genes have been linked to familial PD (Greenamyre and Hastings, 2004; Shen, 2004). Mutations and triplication of α-*synuclein* and mutations in *LRRK2/dardarin* cause autosomal-dominant forms of PD (Greenamyre and Hastings, 2004; Shen, 2004). On the other hand, mutations in *parkin*, *DJ-1*, and *PINK1* cause autosomal-recessive forms of PD (Greenamyre and Hastings, 2004). Studying these genes has been the subject of intense focus with the thought that understanding their function will provide crucial insight into the pathogenesis of the more common, sporadic form of PD. For instance, the linkage of α-*synuclein* with familial PD led to the discovery that α-synuclein is one of the major components of the Lewy body, which provided solid support for a common pathogenic mechanism between the familial and sporadic forms of PD (Dawson and Dawson, 2003). The roles of DJ-1, PINK1, and LRRK2/dardarin in PD are still not clear, but the presence of kinase domains in PINK1 and LRRK2/dardarin suggest that they might be involved in important signaling cascades (Shen, 2004). The function of DJ-1 is not known, but DJ-1 might have chaperone activity and possess important protective properties in the mitochondria when neurons are exposed to oxidative insults (Bonifati et al., 2004).

Oxidative stress and protein metabolism/mishandling seem to be important factors in the pathogenesis of sporadic PD. The critical role of protein metabolism in the pathogenesis of PD is best illustrated by the function of parkin. *parkin* mutations were first described in a group of Japanese patients who developed an autosomal-recessive form of juvenile parkinsonism with clinical symptoms indistinguishable from idiopathic PD (von Coelln et al., 2004). Since its discovery, numerous exon deletions, duplications, and point mutations were found in patients with early-onset PD. In fact, *parkin* mutations can account for up to 40% of early-onset PD (von Coelln et al., 2004). Parkin is an E3 ligase in the ubiquitin system and functions in the targeting of substrates for proteasomal degradation (von Coelln et al., 2004). It is generally believed that accumulation of these substrates due to loss of parkin's activity can lead to disastrous consequences for the survival of dopaminergic neurons (von Coelln et al., 2004). A number of substrates and interactors of parkin have been described. However, no potential interactor has been identified that can regulate parkin's function. Up to now, only posttranslational modification through S-nitrosylation and phosphorylation have been shown to affect the enzymatic activity of parkin (Chung et al., 2004; Yamamoto et al., 2004). In particular, nitrosative stress has been shown to compromise parkin's protective function and provides a common pathogenic pathway for both the familial and sporadic forms of PD (Chung et al., 2004).

In this issue of *Neuron*, Kalia et al., show that BAG5 enhances dopaminergic neuronal degeneration through inhibition of the E3 ligase activity of parkin and the chaperone activity of Hsp70 (Figure 1) (Kalia et al., 2004). Moreover, BAG5 enhances the sequestration of parkin within Lewy body-like aggregates and mitigates parkin-mediated preservation of proteasome function and inhibition of cell death. The human BAG protein family contains six members, and all contain a BAG domain (Takayama and Reed, 2001). The first family member, BAG1, was identified initially as a Bcl binding protein (Bcl-associated athanogene-1) with anti-cell death activity (Takayama and Reed, 2001). Soon, it was found that BAG1 could interact with other proteins, including Hsp70 (Takayama and Reed, 2001). Kalia et al. identified *BAG5* as a gene that is upregulated in the SNc following medial forebrain bundle (MFB) transection (Kalia et al., 2004). Both immunohistochemistry and in situ hybridization confirm that BAG5 is present in dopaminergic neurons in the SNc, and the expression of this gene is selectively increased after MFB axotomy. BAG5 interacts with Hsp70 and interferes with its function to refold misfolded proteins. The activity of BAG5 is dependent on its conserved domains, and mutations of these domains result in the total abrogation of its effect on Hsp70. More importantly, BAG5 was found to directly interact with parkin, even in the absence of Hsp70. The interaction between BAG5 and parkin is specific, and the interaction seems to interfere with the E3 ligase activity of parkin. This is particularly striking, as so far, no proteins have been identified that can interfere with the normal

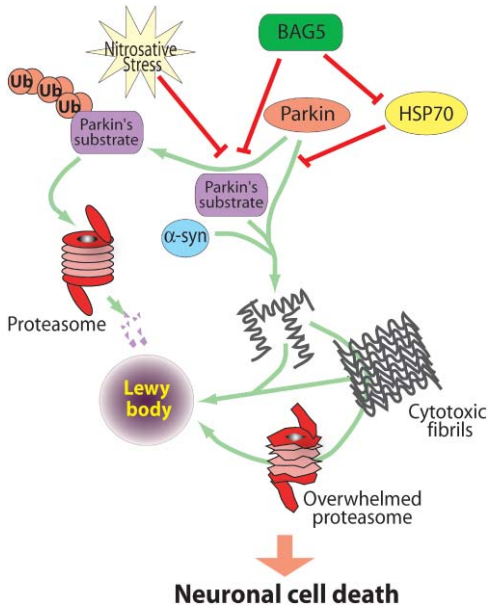

Figure 1. Schematic Diagram Illustrating How BAG5 Can Mediate Its Deleterious Effect on Neuronal Survival

BAG5 interferes with the normal function of parkin and Hsp70, which might ultimately lead to the accumulation of cytotoxic fibrils and formation of protein aggregates. The consequence is the promotion of neurodegeneration.

activity of parkin. BAG5 significantly downregulates parkin's E3 ligase activity. BAG5 also seems to mediate its effect on parkin through the inhibition of the activity of Hsp70 and sequestering parkin into protein aggregates. The mechanism of how BAG5 can compromise parkin's function is illustrated by the experiment that BAG5 enhances proteasomal dysfunction and abrogates parkin's ability to rescue cell death induced by overexpression of α-synuclein and synphilin-1 in the setting of proteasomal inhibition. One of the most significant findings is that overexpression of BAG5 through adenovirus significantly enhances dopaminergic neuronal cell death in SNc induced by MFB axotomy or MPTP-induced toxicity.

The report by Kalia et al. consolidates the importance of the neuroprotective function of parkin and also provides insights into how inhibition of the ubiquitin system and chaperone systems can lead to deleterious consequences in neurons (Figure 1). One of the interesting questions is whether BAG5 acts as a general negative feedback modulator to different E3 ligases in the ubiquitin system or if its activity is specific to parkin. A related family member, BAG1, is known to interact and interfere with the RING finger E3 ligase Siah-1 (Takayama and Reed, 2001). Siah-1 appears to play important roles in the catabolism of synphilin-1 and α-synuclein (Liani et al., 2004). Thus, BAG family members could exert broad effects on the UPS. If this is the case, inhibition of the activity of BAG5 or related family members might provide a possible generalized approach to enhance proteasomal function. Another interesting question is the role of BAG5 in the formation of inclusion bodies. The function of inclusion bodies has been controversial. The conventional view of Lewy bodies in PD is that they are deleterious to the survival of dopaminergic neurons (Olanow et al., 2004). On the other hand, inclusion bodies may just be the byproduct of the process of neurodegeneration (Olanow et al., 2004). However, some studies, especially in the field of polyglutamine-induced neurodegeneration, as in Huntington's disease, have revealed that intracellular inclusions might be the last line of defense against toxic protein species in neurons (Arrasate et al., 2004). BAG5 seems to mediate its effect on parkin, in part, by sequestering it to inclusion bodies and hence ultimately interfering with the normal function of parkin. If the toxic effect of BAG5 is mediated through the formation of protein aggregates, overexpression of it in animal models should result in increased formation of inclusion bodies along with increased neuronal cell death when dopaminergic neurons are exposed to different insults. Finally, the protective function of mutant BAG5 may provide new approaches for the treatment of PD. The family of BAG protein is characterized by the presence of the BAG domain, which possesses the conserved amino acid residues aspartate and arginine. Mutation of these residues seems to provide a dominant-negative effect on BAG5 deleterious properties, and this might present a valuable approach for the gene therapy against the neurodegenerative process of PD.

The work by Kalia et al. clearly illustrates the importance of studying genes that are linked to familial PD, as significant advances in the understanding of the pathogenic mechanisms of PD continue to arise from these studies. The ultimate challenge will be how we can make use of this knowledge in the development of potential new therapeutic strategies for the treatment of PD in the foreseeable future.

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Greasing Transmission: Palmitoylation at the Synapse

Posttranslational modifications such as palmitoylation have the ability to modulate protein localization and function. The reversible addition of the fatty acid palmitate to proteins has been known to occur in neurons for a considerable amount of time and has been noticed to be of particular importance at synapses. In this issue of *Neuron*, Huang et al. and Fukata et al. describe their studies of palmitoyl transferases and how these enzymes specifically catalyze the modification of a number of synaptic proteins, including the postsynaptic scaffolding protein PSD-95.

Once proteins have been synthesized on free ribosomes or on rough ER, they have the potential to be modified by a series of posttranslational additions, such as phosphorylation, ubiquitination, glycosylation, acetylation, and lipid modification. Lipid modifications can include the addition of myristoyl, prenyl, and palmitoyl moieties to the protein. Lipid modifications increase the overall hydrophobicity of the protein and can thereby facilitate the interaction of proteins with cellular membranes. The addition of palmitic acid to proteins in neurons has long been known (Bizzozero, 1997).

More recently, palmitoylation has specifically been shown to play a role in axon pathfinding, neuronal protein trafficking, and the clustering of receptors and associated scaffolding proteins at synapses (El-Husseini and Bredt, 2002). Also, this modification has been well described for a number of presynaptic proteins, including the SNARE proteins VAMP, SNAP-25, and synaptotagmin, which are involved in synaptic vesicle fusion (Veit et al., 1996, 2000). Despite the huge importance of palmitoylation on function in the nervous system, it was only in the last couple of years that the enzymes that catalyze the transfer of palmitate, palmitoyl transferases (or PATs), were described in yeast (Linder and Deschenes, 2004). Palmitate is transferred from Coenzyme A to specific cysteine residues in pretty much any location within the amino acid sequence of a huge variety of proteins. Proteins that are modified range from small cytosolic proteins (e.g., H-ras) to large transmembrane receptors (e.g., GABA-A receptor) to secreted factors (e.g., sonic hedgehog). The cysteine residue to which the C16 fatty acid chain is added is highly reactive and, in fact, this modification can occur in vitro without the need for an enzyme (Veit, 2000). The thioester bond that is formed is labile and thus reversible. It has been shown that regulation of the extent of palmitoylation is required to control a number of processes during development and signaling (El-Husseini and Bredt, 2002). This requirement for specific regulation of palmitoylation has driven the search for the PATs that could modulate processes such as neuronal protein trafficking and synaptic plasticity.

In this issue of *Neuron*, both Fukata et al. (2004) and Huang et al. (2004) describe the properties of recently identified PATs and specifically look at their abilities to palmitoylate synaptic proteins (of which the most prominent is debatably the postsynaptic scaffolding protein, PSD-95). Fukata et al. took a genomic approach and determined all the possible PATs in the mouse genome based on the presence of a DHHC motif. This sequence of amino acids (single-letter code) was determined to be required for activity in yeast PATs and was identified by a forward genetic approach (Linder and Deschenes, 2004). Fukata and coworkers isolated a total of 23 different mouse PATs which they name DHHC-1 through -23. These were then tested for their ability to palmitoylate PSD-95 in transfected nonneuronal cells, in a cell-free assay and in neurons. DHHC-2, -3, -7, and -15 showed the strongest specificity for PSD-95. They further described the differing specificities of these enzymes for other palmitoylated proteins, such as GAP-43, SNAP-25, Lck, $G\alpha_s$, and H-ras.

To determine an effect of palmitoylation of PSD-95, they investigated the ability of the PSD-95 PATs (P-PATs) to change the distribution of PSD-95 from a diffuse pattern to a distinctly perinuclear localization. Since DHHC-15 showed the greatest increase in palmitoylation of PSD-95, they chose this as their favorite and mutated this P-PAT to eliminate its palmitloyl transferase activity. They thereby noticed that mutations at the active site of this enzyme transform these mutants into dominant-negative forms. They proceeded to take advantage of these dominant-negative DHHC-15 molecules to demonstrate that palmitoylation by this P-PAT is indeed important for the punctate localization of PSD-95 and the surface localization of AMPA receptor GluR1.

In contrast, Huang et al. concentrated on one PAT which was cloned by virtue of its interaction with huntingtin and aptly named HIP14 (huntingtin-interacting protein). Due to the presence of a DHHC motif, they surmised that it may be a PAT and described its specificity for a number of synaptic proteins. It was shown to palmitoylate PSD-95, SNAP-25, synaptotagmin I, huntingtin, and GAD65. HIP14 did not demonstrate enzymatic activity toward H-ras, paralemmin, Lck, or synaptotagmin VII. They also proceeded to use the same localization assay as Fukata et al., by which they investigated the correlation of enzymatic activity and the ability to cause proteins to localize to a distinct perinuclear milieu. EM and immunocytochemistry demonstrated localization of HIP14 predominantly to the Golgi apparatus, although immunogold particles were also seen at tubulovesicular organelles in dendrites, axons, and spine necks. Live imaging showed trafficking of HIP14 together with clusters of SNAP25. To determine a requirement of palmitoylation by HIP14 for the punctate distribution of PSD-95, rather than using a dominant-negative, Huang and coworkers used siRNA to effectively knock down the level of HIP14 in neurons (see Figure 1).

While both of these studies elegantly demonstrate the catalysis of palmitoylation of PSD-95 and its require-